Validation of the Soft Embalmed Thiel Cadaver as a High Fidelity Simulator of Pressure during Targeted Nerve Injection

McLeod, Graeme; Zhang, Shengli; Sadler, Amy; Chandra, Anu; Qiao, Panpan; Huang, Zhihong

Published in:
Regional Anesthesia and Pain Medicine

DOI:
10.1136/rapm-2020-102132

Publication date:
2021

Validation of the Soft Embalmed Thiel Cadaver as a High Fidelity Simulator of Pressure during Targeted Nerve Injection

†Graeme A. McLeod, MD FRCA FFPMRCA, ‡Shengli Zhang, †Amy Sadler FRCA, ‡Anu Chandra, ‡Panpan Qiao, ‡ Zihong Huang PhD, and §*Christine Demore PhD.

From the

† Department of Anaesthesia, Ninewells Hospital, NHS Tayside, Dundee, UK

‡ Department of Bioengineering, University of Dundee, UK

§ Sunnybrook Research Institute, Sunnybrook Health Sciences Centre, Toronto, Canada

* Department of Medical Biophysics, University of Toronto, Canada

Address correspondence to:

Graeme McLeod MD, FRCA FFPMRCA

Consultant & Honorary Professor of Anesthesia

Institute of Academic Anesthesia

Ninewells Hospital & University of Dundee, UK.

Email: g.a.mcleod@dundee.ac.uk

=44 1382 632175

The authors declare no conflicts of interest

Funding: The study was funded by Tenovus, Tayside.

Running Head: Fluid injection pressure

Short title: Fluid injection pressures in the Thiel soft embalmed cadaver

Word count

This article has been accepted for publication in *Regional Anesthesia & Pain Medicine* (2021), following peer review, and the Version of Record can be accessed online at http://dx.doi.org/10.1136/rapm-2020-102132.

Abbreviations

COVID 19 Coronavirus

ESRA European Society of Regional Anesthesia

MeSH Medical Subject Headings

PROSPERO International prospective register of systematic reviews

ABSTRACT

Introduction Although administration of regional anaesthesia nerve blocks has increased during the COVID 19 pandemic, training opportunities in regional anaesthesia have reduced. Simulation training may enhance skills, but simulators must be accurate enough for trainees to engage in a realistic way - for example detection of excessive injection pressure. The soft embalmed Thiel cadaver is a life-like, durable simulator that is used for dedicated practice and mastery learning training in regional anaesthesia. We hypothesised that injection opening pressure in perineural tissue, at epineurium and in subepineurium were similar to opening pressures measured in experimental animals, fresh frozen cadavers, glycol soft-fix cadavers and patients.

Methods We systematically reviewed historical data, then conducted 3 validation studies delivering a 0.5ml hydrolocation bolus of embalming fluid and recording injection pressure. First, we delivered the bolus at 12ml.min^{-1} at epimysium, perineural tissue, epineurium, and in subepineurium at 48 peripheral nerve sites on three cadavers. Second, we delivered the bolus at using three infusion rates: 1ml.min^{-1}, 6ml.min^{-1} and 12ml.min^{-1} on epineurium at 70 peripheral nerve sites on five cadavers. Third, we repeated three injections (12ml.min^{-1}) at 24 epineural sites over the median and sciatic nerves of 3 cadavers.

Results Mean (95%) injection pressure was greater at epineurium compared to subepineurium [geometric ratio 1.2 (95%CI: 0.9 – 1.6)], P = 0.04; and perineural tissue [geometric ratio 5.1 (95%CI: 3.7 – 7.0)], P < 0.0001. Mean (95%) injection pressure was greater at 12ml.min^{-1} compared to 1ml.min^{-1} [geometric ratio 1.6 (95%CI: 1.2 – 2.1), P = 0.005]. Pressure measurements were similar in study 3 (P > 0.05 for all comparisons).

Discussion We conclude that the soft embalmed Thiel cadaver is a realistic simulator of injection opening pressure.

INTRODUCTION

Regional anesthesia is recommended for patients either with or suspected coronavirus (COVID) infection, in order to avoid general anesthesia and protect healthcare staff from aerosol generation of respiratory droplets during airway manipulation[1]. However, our department's policy during the pandemic is that anesthesia, including regional block, should be provided by the most experienced anesthesiologist available in order to provide high quality care, and minimise the risk of conversion to general anesthesia. More regional anesthesia has been performed during the pandemic, but not by trainees who have provided support to extended intensive care units.

There is a pressing need to train anesthesiologists in regional anesthesia skills. Both low fidelity[2] and high fidelity simulators[3] show benefit[4], but must be accurate enough for participants to engage in a lifelike way[5] in order to correctly position the tip of the needle relative to the target nerve and experience the realistic ranges of injection pressures. The benefit of high-fidelity replication of pressure, as well other features such as elasticity, resilience and durability, is that it enables deliberate practice[6] - the repetition and successive refinement of performance, a cornerstone of the expert-performance approach to skills training[7].

The soft embalmed Thiel cadaver is the training simulator used at the European Society of Regional Anesthesia (ESRA) cadaver training course in Madrid. It possesses many features of a high-fidelity simulator: physical properties and functionality of cadavers are similar to patients[8]; patient dimensions and anatomical variation reflect clinical practice, and ultrasonic imaging of tissues and needles is realistic[9]. Nerve histology approximates that of humans[10]. Tissue elasticity is retained [11] and confers several benefits. Perineural injection distends then relaxes tissue within the same time-frame as during injection of local anesthetic during clinical nerve block. Ventilation is possible using inflation pressures < 20 cm H_20 (1.96kPa)[12]. Resilience has been consistently demonstrated: hundreds of injections are possible with only minimal tissue disruption [8], needle tracks are not seen and multiple puncture points in skin are not apparent.

Realistic replication of injection pressure at tissue interfaces, as well as within nerves, is particularly important for detecting the precise location of the needle tip because high subperineural fluid injection pressure is a recognized factor in the genesis of nerve injury[13] and fluid injection pressure > 103kPa (15psi) is a sensitive means of differentiating between epineural contact [14] and perineural needle tip placement in patients [14; 15].

Cadaver validation is also essential for research. New block techniques may be tested and imaged within a safe environment; new ultrasound and needle technologies developed and tested; and device regulatory studies conducted without the need for animal experimentation.

Our initial objective was to review the literature pertaining to measurement of injection pressure in anaesthetised dogs[16; 17], pigs[18; 19], fresh frozen cadavers[20-22], phenol/glycol cadavers[23; 24] and patients[14; 15].

We then conducted three separate studies in order to validate characteristics of pressure measurement observed in the aforementioned studies; notably discrimination between needle tip tissue location[14-16], and correlation between fluid injection rate and opening pressure[16; 25; 26]. Therefore, the objective of the first study was to compare opening injection pressure, on epimysium, in perineural tissue, at epineurium, and in subepineurium. The objective of the second study was to validate the linear relationship between opening injection pressure and flow rate. We also conducted a third study in order to investigate whether opening injection pressure measurement was repeatable at the same site.

METHODS

We reviewed the literature pertaining to measurement of injection pressure in animals, cadavers and patients. We searched, MEDLINE, Embase, CINAHL databases, registers of ongoing trials (www.clinicaltrials.gov and www.controlled-trials.com) in order to identify all published papers investigating pressure measurement at nerves. We used the guidelines for systematic reviews provided by the International prospective register of systematic reviews[27] (PROSPERO). Our review question was: "does in-line pressure measurement differentiate between needle tip position?" We searched the Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library, EMBASE, MEDLINE, EBSCOhost and Web of Science.

We used the Medical Subject Headings (MeSH) search term 'nerve block', with free text 'pressure', 'ultrasonography', 'peripheral nerves', 'nerve', 'fascicle', 'epimysium', 'perineural', 'epineurium', 'perineurium', 'extraneural' and 'intraneural'. One author (GM) performed the search and collated the studies. The types of studies included were randomized trials and observational studies comparing in-line opening pressure measurement either in perineural tissue, at epineurium, within subepineurium or in subperineurium (within fascicles). Our primary outcome was opening pressure. Secondary outcomes included covariates such as injection model (animal, cadaver or patient); nerves targeted; infusion rate; and the relative proportion of injections < or ≥ 15 psi (103kPa). Data was expressed as mean (SD) and range noted where available.

We then conducted three experiments at the Centre for Anatomy and Human Identification, University of Dundee, UK in order to validate opening pressure measurements on human soft embalmed cadavers. Written permission was given by the Thiel cadaver advisory committee. The senior anatomy technician chose twelve cadavers that had completed at least six months soft embalming. The first cadaver was used for training the operators, and ensuring they were comfortable with needling and ultrasound techniques before starting the study. The remaining 11 cadavers were distributed amongst three studies.

For all three studies, a 100mm 21g nerve block needle (Stimuplex, B.Braun, Melsungen, Germany) was connected in-line to a fluid pressure sensor (P-MAT, PendoTech, New Jersey) with a measurement range up to 517kPa (75 psi), and to a 30ml syringe driven by an automated infusion device (PHD Ultra, Harvard Instruments, Holliston, MA). The infusion device was programmed to deliver a 0.5ml bolus of Thiel embalming fluid at controlled rate. We used a 0.5ml Hydrolocation bolus in our routine clinical practice in order to confirm the position of the tip of the block needle. The microultrasound imaging system and P-MAT system were time synchronised, and pressure data was measured at a rate of 0.5 Hz.

Study 1. Injection fluid pressure versus needle tip position

The primary objective of the first study was to compare opening fluid injection pressure when the needle tip was within perineural tissue, on contact with epineurium and within subepineurium. We hypothesized, based on our previous work on anaesthetized pigs[28], that opening fluid injection pressure at epineurium was greater than that at perineural and subepineural needle locations. We also decided to measure opening pressure on indentation of the epimysium of muscle. The epimysium is distinct on ultrasound imaging, and provides resistance to needle penetration, but is rarely evaluated. Thus, we anticipated that our study would provide data on all significant tissues potentially encountered by needles during cadaver training and research.

We imaged and targeted 48 upper and lower limb nerve sites on three soft-embalmed Thiel cadavers (Nos. 2 – 4), with the order of injection randomised by computer software to the left and right sides of eight nerves and two operators, a consultant and a fellow in regional anesthesia (Fig 1). Nerves were imaged using a 3 to 8MHz linear ultrasound transducer connected to a Zonare Ultra ultrasound machine (Zonare, Mountain View, CA). This is the transducer dedicated to imaging anatomical structures in Thiel cadavers.

The nerves imaged were: the C5 and C6 ventral nerve roots, mid-forearm median and ulnar nerves, the femoral nerve, popliteal tibial nerve, and mid and proximal thigh sciatic nerves. The C5 and C6 ventral nerve roots were identified medial to the scalenus medius muscle. Forearm nerves were imaged in the fascial plane between the superficial and deep flexor muscles. The median nerve was located medial to the flexor carpi radialis, and the ulnar nerve lateral to the flexor carpi ulnaris muscle. The femoral nerve was imaged in the groin beneath the sartorius muscle and fascia iliaca. The popliteal tibial nerve and sciatic nerve sites were located medial to biceps femoris and lateral to semitendinosus and semi-membranosus. All needles were inserted using an in-plane approach and penetrated the aforementioned muscle groups.

We indented epimysium and epineurium with needles using the same method described by Gadsden et al[14; 15]. The needle tip position was confirmed on ultrasound images by both operators, and the times of injection recorded by an independent research assistant.

We were aware from previous studies[29] that direct placement of needles against epineurium is difficult; nerves are pushed backwards and rotate, and needles tend to veer tangentially. Therefore, if the needle or the nerve slipped away during injection, the operator repeated the procedure, by removal of the needle and reinsertion through skin.

Study 2. Injection fluid pressure versus infusion rate

The objective of the second experiment was to investigate the effect of infusion rate on fluid injection pressure when the needle tip was in contact with epineurium. The flowchart is shown in Fig 1. Two operators scanned nerves using a 22 - 45MHz UHF48 microultrasound transducer attached to a FUJIFILM VisualSonics Vevo MD ultrasound machine (VisualSonics, Amsterdam, Netherlands) that is used clinically for neonatology, vascular imaging and dermatology. This imaging system provides high quality images of structures up to 2cm from the skin, so restricted us to imaging seven superficial nerves on both sides of five soft embalmed Thiel cadavers (Nos. 5 – 9). Nerves included

the C5 and C6 nerve roots, axillary median and radial nerves, forearm median and ulnar nerves, and the popliteal tibial nerve. The approach to the nerve roots, forearm and femoral nerves was the same as that employed in the first study. The axillary median and radial nerves were visualized in the axilla, lateral and inferior respectively to the axillary artery. The latter was visualized as a small anechogenic area.

The operators consisted of an experienced regional anesthesiologist and a PhD student trained previously in regional anesthesia nerve block on the soft embalmed Thiel cadaver. The PhD student was trained repeatedly on cadaver 1 by the primary investigator until she had demonstrated competencies equivalent to trainee anesthesiologists at the end of basic training, defined by the Royal College of Anaesthetists 2010 curriculum.

The procedure consisted of percutaneous insertion and indentation of the epineurium of the targeted nerve as described above. The pressure measurement set up was the same as in study 1. We used three infusion rates, 1ml.min^{-1}, 6ml.min^{-1} and 12ml.min^{-1}, in order to deliver the 0.5ml fluid bolus. However, the number of blocks was restricted by the number of cadavers and superficial injection sites we had available. Therefore, we decided to randomize to 28 injections at 6ml.min^{-1} and 28 injections at 12ml.min^{-1}, in order to maximise data from clinically relevant flow rates. We also performed 14 control injections at a subclinical flow rate of 1 ml.min^{-1} over 30s. This acted as a control measure that gave us three widely separated flow rates to chart against opening pressure. These flow rates mirrored those used in an anaesthetised pig study with 40MHz microultrasound to identify nerves clearly[19], and thus offered us the opportunity to directly validate the relationship between flow and pressure in cadavers against that observed in anaesthetised pigs.

Study 3. Repeated injection

We conducted a third study to evaluate the effect of repeated injection by the same operator on opening pressure measurements at epineurium. The pressure recording set up was the same as that used in the two previous studies. The flowchart is shown in Fig 1. The order of injections were randomised equally between the proximal and distal sites of median and sciatic nerves on both sides of 3 cadavers (Nos. 10 – 12), for a total of 24 procedure sites. The proximal median nerve site lay between pronator teres and brachialis muscle adjacent to brachial artery, and the mid-arm site was medial to flexor carpi radialis. Needle insertion sites were approximately 15 cm apart. The proximal sciatic nerve site was identified on ultrasound inferior to a line intersecting the greater trochanter and ischial tuberosity. The distal site corresponded to the apex of the popliteal fossa. At both sites the sciatic nerve lay between the biceps femoris laterally and the semi-tendinosus and semi-membranosus medially.

A single experienced operator inserted a 100mm 21g nerve block needle (Stimuplex, B.Braun, Melsungen, Germany) from the lateral side through biceps femoris towards the nerve target and indented the epimysium and nerve epineurium in the same fashion as preceding studies. A 0.5ml hydrolocation dose was injected and pressure trace recorded. Epineural injections were repeated three times as separate procedures. On each occasion the needle was withdrawn completely from the skin then reinserted. On the third occasion the operator attempted to penetrate the epineurium with the needle, entered the nerve and performed the injection. If the operator had difficulty recognizing intraneural swelling, then intraneural injection was repeated.

The secondary objectives of all studies were to investigate the effect of nerve site, cadaver, left and right sides, and operators on fluid injection pressure.

Statistical Analysis

The maximum pressure measured over the duration of the injection was taken as the opening injection pressure. The distribution of the opening pressure data was assessed using the D'Agostino-

Pearson omnibus K^2 test (Graph Pad Prism 8.2.0, GraphPad Software, San Diego CA) and we used quartile-quartile (Q-Q) plots to visualise fit to normal and log-normal distributions. We log-converted pressure data and used a mixed effects regression model to analyse paired data and model our covariates against fascial, epineural and intraneural fluid injection pressures. All pressures were considered as intention-to treat data and used for analysis. Items were compared using individual comparison hypothesis tests. Results are expressed as geometric mean (95%CI).

For Study 1, power analysis was based on comparison of paired, dependent fluid pressure data at epineurium and during intraneural injection. From a previous anaesthetised pig experiment[28] we assumed an effect size of 0.5, and two-tailed test with α error = 0.05 and β error = 0.90. We calculated (G*Power, University of Dusseldorf) that we needed at least 22 paired measurements. Given that 8 injections were performed on each side of the cadaver, we used three cadavers and administered 48 injections.

Study 2 was powered on the correlation of three injection rates. We assumed an effect size of 0.5, and two-tailed test with α error = 0.025 and β error = 0.90. We used α error = 0.025 because we were comparing three groups. We needed 68 injections and therefore conducted 70 injections on 5 cadavers (7 injections per side per cadaver).

In order to power study 3, we hypothesised that there would be no difference in opening pressure during repeated injections at epineurium. We powered the study based on the difference between means being less than half the common standard deviation (equivalent to an effect size = 0.5). Using a two-tailed test with α error = 0.025 and β error = 0.90 for 3 paired groups, we calculated that we needed at least 52 injections. We therefore, randomised to a total of 72 injections on epineurium. This consisted of 24 injections (two per limb per side of three cadavers) repeated three times.

RESULTS

Table 1 summarises studies measuring pressure at subepineural sites in dogs, pigs, fresh frozen and soft fix cadavers, as well as pressures encountered on needle-nerve contact on patients.

Table 1. Summary of pressure studies in dogs, pigs, fresh frozen cadavers, phenol/glycol cadavers and patients. Data presented as mean (SD) and [range] if available from published article.

						Subperineural		Subepineural		Epineural		Perineural		
Author	Species/ model	Nerve(s)	Inf. rate (ml/min)	Vol. (ml)	Inj (n)	Mean (SD) [range] kPa	Mean (SD) [Range] psi	Mean (SD) [range] kPa	Mean (SD) [Range] psi	Mean (SD) [range] kPa	Mean (SD) [range] psi	Mean (SD) [range] kPa	Mean (SD) [range] psi	Comments
Hadzic et al [16]	Dog	Sciatic	4	4	14	150 (117) [21- 310]	22 (17) [3 - 45]					21 (6)	3 (1)	Intraneural: 57% > 172kPa (25psi) All perineural < 28kPa (4psi)
Kapur et al [17]	Dog	Sciatic	4	4	20			207 (48) [14 - 262]	30 (7) [2 - 38]			55 (21)	8 (3)	Intraneural: 40% > 75kPa (11psi)
Lupu et al [18]	Pig	Median - forearm	15	10 - 20	20			35 (20) [28 - 35]	5 (3) [2 - 9]					100% < 103kPa (15 psi)
Altermatt et al [19]	Pig	Brachial plexus Femoral	4		24			48 (55) [0 - 221]	7 (8) [0 - 32]					20% > 103kPa (15 psi)
Orebaugh et al [20]	Fresh cadaver	Cervical roots	20	5	8			337 (69) [255 - 455]	49 (25) [37 - 66]					100% > 103kPa (15 psi)
Ross et al [21]	Fresh cadaver	Cervical roots Peripheral nerves (MC and ulnar in axilla, median and radial in arm, femoral)	20	5	14	415 (119) 225 (97)	60 (17) 33 (14)					148 (59)	22 (9)	
Vermeyleen et al	Fresh cadaver	Femoral Saphenous Sciatic Tibial Common peroneal	10	10	10 0			157 (33) 152 (18) 172 (34) 151 (34) 181 (30)	22 (5) 22 (3) 25 (5) 22 (5) 26 (4)			27 (6) 38 (12) 31 (16) 43 (14) 31 (12)	4 (1) 5 (2) 5 (2) 6 (2) 6 (2)	100% > 103kPa (15 psi)
Krol et al [23]	Phenol cadaver	Median Radial Ulnar	6	1	60			206 (65) 191 (60) 125 (49)	29 (9) 27 (9) 18 (7)				7 (3) 8 (3) 7 (2)	
Krol et al [24]	Phenol cadaver	Cervical trunk Supraclavicular Infraclavicular	6	1	30			218 (42) 168 (105) 164 (67)	31 (6) 24 (15) 23 (10) 23 (9)			6 (2) 9 (6) 5 (3) 6 (2) 6 (2) 7 (2)		87% > 103kPa (15 psi)

		Sciatic Peroneal Tibial						158 (62) 138 (25) 120 (51)	20 (4) 17 (7)					
Gadsden et al [15]	Patient	Femoral	10	1	20					103 (14) [83 - 131]	15 (2) [12 - 19]			90% > 103kPa (15 psi)
Gadsden et al [14]	Patient	Interscalene	10	1	36					145 (48)	21 (4)			97% > 103kPa (15psi)

Study 1. Needle insertion and injection at fascia, epineurium and in subepineurium.

Fig 2 shows representative ultrasound images of needle insertion, tissues and target nerve.

The median [range time from first embalming was 6 [6 – 7] months for cadavers 2 – 4. Injections were performed at 48 nerve sites. We failed to enter nerves on 3 occasions. On 11 occasions the nerve rotated and the needle was directed tangentially. These injections were repeated successfully giving 56 pressure measurements at epineurium for analysis. Injection at epimysium was identified on 84 occasions.

Pressure data had a log-normal distribution (Fig. 3). All perineural pressures were < 103kPa (15psi). Pressures > 103kPa (15psi) was recorded on 36 (64%) occasions at epineurium, 22 (49%) occasions in subepineurium and on 46 (55%) occasions at epimysium. Opening pressure was higher at epineurium compared to perineural tissue [geometric ratio 5.1 (95%CI: 3.7 – 7.0)], $P < 0.0001$, epimysium [geometric ratio 1.3 (95%CI: 1.0 – 1.6)], $P < 0.006$, and subepineurium [geometric ratio 1.2 (95%CI: 0.9 – 1.6)], $P = 0.04$ (Fig 3B and C). Opening pressure in subepineurium was greater than that in perineural tissues [geometric ratio 4.2 (95%CI: 3.0 – 5.0), $P < 0.0001$. There was no difference in opening pressure between nerves (Fig 3D)

Superimposed pressure/time plots at epimysium, in perineural tissue, on epineurium and in subepineurium are over the mid-sciatic nerve, femoral nerve, ulnar nerve, and popliteal nerve. Images demonstrate variation in relative pressures measured at different interfaces. For example image A shows a rank order of pressure: epineurium > epimysium> subepineurium > perineural tissue, whereas image B shows higher pressures during subepineural injection. Baseline pressure varied between 0.5 and 1.5psi, but remained constant for subsequent injections, including subepineural injection, and returned to pre-injection levels on tissue relaxation.

Study 2. Fluid injection flow rate

Median [range] time from first embalming was 6 [6 – 8] months for cadaver nos. 5 – 9. Fig. 4 shows microultrasound images of a block needle approaching and indenting the right interscalene C5 and C5 ventral nerve roots of cadaver no. 8. The image depth is 15.5mm, and thus nerve roots appear larger than seen on standard ultrasound images.

Seventy injections were performed with the needle tip on the epineurium. Eight out of 70 (11%) epineural pressures, were > 103kPa (15psi). The distributions of pressure measurements were log-normal (Fig 5A and 5B). Mean pressure was greater using a flow rate of 12ml.min^{-1} compared to a flow rate of 1ml.min^{-1} [geometric ratio 1.6 (95%CI: 1.2 – 2.1), P = 0.005].

Study 3. Repeated injection

Median [range] time from first embalming was 7 [7 – 8] months for cadaver nos. 10 – 12. At 22 out of 24 sites we successfully indented epineurium three times. At two sites successfully indented epineurium on 2 occasions, giving 70 epineural injections for analysis. Opening pressure at epimysium was recorded on 68 occasions. We attempted 30 intraneural injections. Six were repeated at the same site, because the operator had difficulty recognising uniform nerve swelling expected following intraneural injection.

Sixty-five (93%) epineural pressures were > 103kPa (15psi). Data were log-normal. There was no difference in pressure between repeated injections on epineural contact with median and sciatic nerves, at either proximal or distal sites (all P-values > 0.05), Fig 5C.

Fluid pressure was greater at epineurium than epimysium [geometric ratio 1.3 (95%CI: 1.1 – 1.5), P < 0.001] and greater at epineurium than subepineural injection [geometric ratio 1.8 (95%CI: 1.5 – 2.2), P = 0.002].

Secondary end-points

Mixed models analysis showed no statistical difference in fluid pressure between cadavers, nerves, side of injection, or between injection sites in studies 1, 2 and 3 (all P-values > 0.05).

DISCUSSION

Together, our studies show that the soft embalmed Thiel cadaver is high fidelity simulator of fluid injection pressure. As in animals and fresh cadavers, opening pressure was consistently higher at epineurium than fluid pressure at perineural tissue and during subepineural injection. Opening injection pressure increased with flow rate. Opening pressure on epineurium did not change with repeated injection. No differences were seen in fluid injection pressure between nerves, sites of injection, cadavers, sides and operators.

The magnitude and range of pressures varied at each anatomical location within our three experiments, but remained within the values obtained from previous studies conducted in unembalmed cadavers[20-22], glycol/phenol preserved cadavers[23; 24]; pigs[18; 19], dogs[16; 17] and patients[14; 15] (Table 1). Indeed, subepineural pressures were closest to those obtained in pigs[28] and epineural pressures were closest to those obtained in patients[14; 15].

We used a 0.5 mL injection bolus to mimic bolus test doses, a smaller volume than prior studies, and may explain the smaller fraction of epineural pressures that exceed the 103kPa (15 psi) threshold measured in patients[14]. Nevertheless, we feel that our study is clinically relevant because a 0.5mL volume is commonly used as a hydrolocation dose by regional anesthesiologists, and, imaged by microultrasound, is associated with marked traumatic changes in nerve structure[29]. We intend to use a larger bolus in future studies in order to examine the dose response of cadaver tissue.
There was a considerable overlap of pressure data between anatomical locations. Overall the rank order of fluid pressure at tissue may be summarised as epineural > subepineural = epimysium > perineural. The proportion of pressures distal than our 15psi (103kPa) threshold derived from Gadsden et al[14] varied between 11% and 89% for epineural injection and between 23% and 51% for subepineural injection. These findings agree with those of Kapur[20] who encountered pressures < 83kPa (12psi) in 60% of dogs during subepineural injection.

Baseline pressure at the start of perineural, epineural and subepineural injection was raised by between 0.5psi to 1.5 psi. We suspect that small fluid retention occurred in tissues but was not visible on ultrasound images. Importantly, baseline fluid injection pressure did not rise with repeated injections, indicating that fluid was dissipating away from the site of injection. We cannot explain however, why subepineural injection started from a slightly raised baseline as we would not expect 0.5ml extraneural injection to influence intraneural injection. Irrespective, use of repeated injections replicated our clinical practice of delivering hydrolocation doses in order to gauge the position of the needle tip relative to tissue.

Our findings may be explained by examination of the distribution of data. We demonstrated that fluid pressures at all anatomical sites in all studies followed the lognormal distribution, a scattering of data common in nature that describes many biological processes. In order to confirm this finding, we log converted all pressure data and subsequently demonstrated a normal distribution from all anatomical sites (Fig 3).

The lognormal distribution explains the wide variation in pressures observed in all studies (Table 1) It counters the hypothesis presented in correspondence[30] that pressure automatically differentiates between subperineural injection and subepineural injection. Our results emphasise the need to present the distribution and range of pressure data as well as the mean (SD) or median [IQR].

The lognormal distribution may be understood by consideration of the elasticity of tissue in homogenous, isotropic tissue. Force or stress applied perpendicular to tissue induces a strain or relative displacement that is linear over a very small increment in force (Hooke's Law)[31]. With greater strain, stress progressively increases in an exponential manner and tissues fracture. The mathematical relationship, however, is not exact because soft tissue is not homogenous but visco-elastic, poroelastic, anisotropic and contractile and is altered by age and disease. There is also some

flow and resistance to flow affecting the pressures, that becomes complex with the tissue displacement or fracture. We therefore recommend log-conversion of injection pressures for statistical analysis and comparison between groups.

We use soft-embalmed Thiel cadavers as simulators for teaching, training & research. The Thiel embalming process was developed as a method to preserve tissues while retaining elasticity joint motion, and lifelike colouring of tissues[32]. The cadavers are soaked for 6 months in large vats containing water-based solutions of salts and acids, a process which makes the cadavers aseptic[33]. The cadavers can be stored for up to 3 years, and are regulated by the Anatomy Act 2006 (Scotland). The functionality of the soft embalmed cadaver has enabled us to change the way we train in regional anesthesia. We use dedicated, repetitive practice and mastery learning [11] and quantitative measurement of learning curves[3] using validated task and error checklists [11] .

Weaknesses of study

The principal weakness of the study is that we used a commercial pressure monitor used in previous studies that sampled at only 0.5Hz. There is a risk with such response rates that true peak pressures are missed. We have adapted a similar commercial pressure monitor to gather data at 10Hz and will use this in future studies. Moreover, accurate needle-nerve contact was restricted by the resolution of the 5 to 12MHz ultrasound transducers, and accounts for the need for 11 repeated injections in study 1 and 6 extra subepineural injections in study 3. Interestingly, we had no technical issues in study 2 because we were using a 22 - 45MHz ultrasound transducer that offered better resolution of the nerve and permitted better needle orientation (Fig. 2 and Fig. 4) and needle tip placement to align the needle tip perpendicular to the epineurium. Microultrasound, defined as ultrasound transducer frequencies ≥ 30MHz is now clinically available (Visualsonics, Toronto, Canada) and is used to image neonates and children. Use of high frequencies means that only tissue lying within 15mm from the skin surface can be visualised with greater resolution than standard 10 to 15MHz

transducers normally used in regional anaesthesia. Loss of muscle mass in the elderly means that many peripheral nerves are visible at these distances within soft embalmed cadavers. Our images in Fig 4 have a depth of 13.5mm and provide an enlarged representation of the interscalene nerve roots. We feel that this technology would enhance paediatric regional anaesthesia.

Pressure may be measured at the tip of the needle but this technology is not yet available for clinical use[34]. In the meantime, we hypothesise that the detection of subepineural injection using in-line pressure monitoring is associated with a substantial false negative rate, albeit that, according to our results, detection of epineural contact is probably more accurate.

Conclusions

A picture is emerging of a life-like simulator that possesses not only similar elasticity to patients and imitates the flow of local anaesthetic around nerves but also replicates the pressures encountered in patients, animals and fresh cadavers during epineural and subepineural injection. Overall, our evidence suggests that the soft embalmed cadaver provides a realistic simulation environment that can be used for a variety of purposes including simulation-based training of nerve block to anesthesiologists. We see many potential benefits to availability of a high-fidelity simulator include learning skills before undertaking them in supervised clinical practice; testing proficiency as anesthesiologists move from novice learners to experts; practising non-technical skills out-with the operating theatre; evaluating training approaches; research in developing new block techniques; developing and evaluating new technology; and offering a model for device regulation without animal experimentation.

Author contributions

GM and CD designed the study and wrote the paper

ZH was grant holder and supervised SZ's and PQ's PhD studies

SZ, AS, AC and PQ conducted the study

GM and CD supervised AC's PhD studies

GM supervised AS, regional fellow

ACKNOWLEDGEMENT

We wish to thank Visualsonics, Amsterdam, Netherlands for loan of a VEVO MD microultrasound machine

COMPETING INTERESTS

Professor McLeod is a member of the European regional anesthesia scientific advisory board of BBraun/Philips. He has received funding for conducting studies and presentation of research at the European Society of Regional Anaesthesia.

1. Uppal V, Sondekoppam RV, Landau R, El-Boghdadly K, Narouze S, Kalagara HKP. Neuraxial anaesthesia and peripheral nerve blocks during the covid-19 pandemic: A literature review and practice recommendations. *Anaesthesia* 2020;75:1350-1363.
2. Chuan A, Lim YC, Aneja H et al. A randomised controlled trial comparing meat-based with human cadaveric models for teaching ultrasound-guided regional anaesthesia. *Anaesthesia* 2016;71:921-9.
3. McLeod GA, McKendrick M, Taylor A et al. An initial evaluation of the effect of a novel regional block needle with tip-tracking technology on the novice performance of cadaveric ultrasound-guided sciatic nerve block. *Anaesthesia* 2020;75:80-88.
4. Chuan A. Education and training in ultrasound-guided regional anaesthesia and pain medicine. *Curr Opin Anaesthesiol* 2020;33:674-684.
5. Ericsson KA. Acquisition and maintenance of medical expertise: A perspective from the expert-performance approach with deliberate practice. *Acad Med* 2015;90:1471-86.
6. Ahmed OMA, Azher I, Gallagher AG, Breslin DS, O'Donnell BD, Shorten GD. Deliberate practice using validated metrics improves skill acquisition in performance of ultrasound-guided peripheral nerve block in a simulated setting. *J Clin Anesth* 2018;48:22-27.

7. Ericsson KA. Deliberate practice and the acquisition and maintenance of expert performance in medicine and related domains. *Acad Med* 2004;79:S70-81.
8. Munirama S, Eisma R, Columb M, Corner GA, McLeod GA. Physical properties and functional alignment of soft-embalmed thiel human cadaver when used as a simulator for ultrasound-guided regional anaesthesia. *Br J Anaesth* 2016;116:699-707.
9. Munirama S, Joy J, Columb M et al. A randomised, single-blind technical study comparing the ultrasonic visibility of smooth-surfaced and textured needles in a soft embalmed cadaver model. *Anaesthesia* 2015;70:537-42.
10. Chandra A, Eisma R, Felts P et al. The feasibility of micro-ultrasound as a tool to image peripheral nerves. *Anaesthesia* 2017;72:190-196.
11. McLeod G, McKendrick M, Taylor A et al. Validity and reliability of metrics for translation of regional anaesthesia performance from cadavers to patients. *Br J Anaesth* 2019;123:368-377.
12. Eisma R, Gueorguieva M, Immel E et al. Liver displacement during ventilation in thiel embalmed human cadavers - a possible model for research and training in minimally invasive therapies. *Minim Invasive Ther Allied Technol* 2013;22:291-6.
13. Hadzic A, Vloka JD, Claudio RE, Hadzic N, Thys DM, Santos AC. Electrical nerve localization: Effects of cutaneous electrode placement and duration of the stimulus on motor response. *Anesthesiology* 2004;100:1526-30.
14. Gadsden JC, Choi JJ, Lin E, Robinson A. Opening injection pressure consistently detects needle-nerve contact during ultrasound-guided interscalene brachial plexus block. *Anesthesiology* 2014;120:1246-53.
15. Gadsden J, Latmore M, Levine DM, Robinson A. High opening injection pressure is associated with needle-nerve and needle-fascia contact during femoral nerve block. *Reg Anesth Pain Med* 2016;41:50-5.
16. Hadzic A, Dilberovic F, Shah S et al. Combination of intraneural injection and high injection pressure leads to fascicular injury and neurologic deficits in dogs. *Reg Anesth Pain Med* 2004;29:417-23.
17. Kapur E, Vuckovic I, Dilberovic F et al. Neurologic and histologic outcome after intraneural injections of lidocaine in canine sciatic nerves. *Acta Anaesthesiol Scand* 2007;51:101-7.
18. Lupu CM, Kiehl TR, Chan VW, El-Beheiry H, Madden M, Brull R. Nerve expansion seen on ultrasound predicts histologic but not functional nerve injury after intraneural injection in pigs. *Reg Anesth Pain Med* 2010;35:132-9.
19. Altermatt FR, Cummings TJ, Auten KM, Baldwin MF, Belknap SW, Reynolds JD. Ultrasonographic appearance of intraneural injections in the porcine model. *Reg Anesth Pain Med* 2010;35:203-6.
20. Orebaugh SL, Mukalel JJ, Krediet AC et al. Brachial plexus root injection in a human cadaver model: Injectate distribution and effects on the neuraxis. *Reg Anesth Pain Med* 2012;37:525-9.
21. Ross S, Edwards K, McFadden K, Bigeleisein P, Orebaugh S. Pressures of injection in a cadaver model of peripheral nerve blockade. *Journal of Anesthesia & Clinical Research* 2014;5: 1000445.
22. Vermeylen K, Hermans M, Soetens F et al. Opening injection pressure is higher in intraneural compared with perineural injections during simulated nerve blocks of the lower limb in fresh human cadavers. *Reg Anesth Pain Med* 2017;42:362-367.
23. Krol A, Szarko M, Vala A, De Andres J. Pressure monitoring of intraneural an perineural injections into the median, radial, and ulnar nerves; lessons from a cadaveric study. *Anesth Pain Med* 2015;5:e22723.
24. Krol A, Vala A, Phylactides L, Szarko M, Reina MA, De Andres J. Injection pressure mapping of intraneural vs. Perineural injections: Further lessons from cadaveric studies. *Minerva Anestesiol* 2018;84:907-918.

25. Patil JJ, Ford S, Egeler C, Williams DJ. The effect of needle dimensions and infusion rates on injection pressures in regional anaesthesia needles: A bench-top study. *Anaesthesia* 2015;70:183-9.
26. Quadri C, Saporito A, Capdevila X. Real-time continuous monitoring of injection pressure at the needle tip for peripheral nerve blocks: Description of a new method. *Anaesthesia* 2018;73:187-194.
27. Booth A, Clarke M, Dooley G et al. The nuts and bolts of prospero: An international prospective register of systematic reviews. *Syst Rev* 2012;1:2.
28. Chandra A, Yan J, Soenjaya Y, Demore C, Columb M, McLeod G. Identifying the needle-tip position during regional anaesthesia with needle-tip pressure at different flow rates. *Br J Anaesth* 2018;121:e30-e31.
29. Chandra A, McLeod G, Yan J, Soenjaya J, Morningstar B, Demore C. In vivo microultrasound visualisation of nerve trauma due to regional anaesthesia needle insertion and injection. *2018 IEEE International Ultrasonics Symposium (IUS);101109/ULTSYM20188579830* 2018.
30. Selander DE. Re: Combination of intraneural injection and high-injection pressure leads to fasicular injury and neurologic deficits in dogs. *Reg Anesth Pain Med* 2005;30:308-9; author reply 309-10.
31. Wells PN, Liang HD. Medical ultrasound: Imaging of soft tissue strain and elasticity. *J R Soc Interface* 2011;8:1521-49.
32. Thiel W. [the preservation of the whole corpse with natural color]. *Ann Anat* 1992;174:185-95.
33. Eisma R, Wilkinson T. From "silent teachers" to models. *PLoS Biol* 2014;12:e1001971.
34. Saporito A, Quadri C, Capdevila X. The ability of a real-time injection pressure monitoring system to discriminate between perineural and intraneural injection of the sciatic nerve in fresh cadavers. *Anaesthesia* 2018;73:1118-1122.

Fig 1 Flow chart. Flow diagrams for the three studies replicating peripheral nerve blocks on Thiel embalmed cadavers showing selection of nerves for intervention sites. C5, C6: ventral cervical nerve roots. Med, Uln: mid-forearm median and ulnar nerves. Fem: femoral nerve. Tib-P: popliteal tibial nerve. Sci-M, Sci-U: mid and upper (proximal) sciatic nerve. Med Ax, Rad Ax: axillary median and radial nerves.

Fig 2. Representative ultrasound images of the right neck of cadaver 3 taken with a 3 to 8MHz transducer over the ventral C5 and C6 interscalene nerve roots. Images are cropped to a depth of 25mm. Image pairs are shown without (left) and with (right) annotation indicating needle position (3 arrowheads), C5 and C6 nerve roots outlined in yellow, scalenus medius (SM) outlined in blue, and scalenus anterior (SA) outlined in green. Images A and B show the needle tip approximately 3 mm from C6. Images C and D show the needle gently contacting epineurium. Images E and F show the needle tip within the subepineurium of C6 and the nerve has expanded secondary to subepineural injection.

Fig 3. Injection peak pressure measurement from Study 1 with needle tip at fascia, at epineurium and in the subepineurium. Data was log-normal. Quantile-quantile (Q-Q) plot of logged pressure (A) follows a straight line and logged data show normal distribution (B). Pressure at epineurium greater than at other anatomical locations (C). Pressures follow rank order: epineurium > subepineurium = epimysium > perineural. Differences between pressures at anatomical interfaces over range of peripheral are consistent (D).

Fig 4 Ultrasound images of the right interscalene C6 ventral nerve root in soft embalmed Thiel cadaver no. 8, imaged using a 22- 45Mz microultrasound transducer. The scale on the right-hand side is from 0.5 to 15.5mm. Image pairs are show without (left) and with (right) annotation

indicating needle position (arrowheads), C5 and C6 nerve roots outlined in yellow, and scalenus anterior muscle outlined in green. Images A and B shows the needle approaching the nerve. The tip is approximately 2 mm from the epineurium. Images C and D shows the needle touching the epineurium. Images E and F shows the needle tip indenting the epineurium.

Fig 5. Injection peak pressures measurement from study 2 at epineurium using flow rates of 1ml.min^{-1}, 6ml.min^{-1} and 12ml.min^{-1} displayed with (A) linear scale and (B) following log conversion. Pressure measurements show a difference in pressure between a flow rate of 1ml.min^{-1} and 12ml.min^{-1}, P = 0.005. Effect of repeated needle insertion in study 3 (C). No difference in opening injection pressure with repeated injection at each nerve or injection site (all P-values > 0.05).

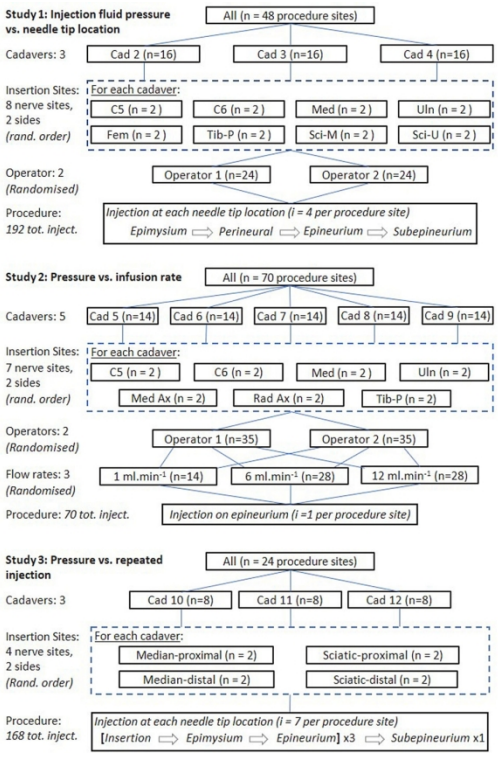


Fig 1 Flow chart. Flow diagrams for the three studies replicating peripheral nerve blocks on Thiel embalmed cadavers showing selection of nerves for intervention sites. C5, C6: ventral cervical nerve roots. Med, Uln: mid-forearm median and ulnar nerves. Fem: femoral nerve. Tib-P: popliteal tibial nerve. Sci-M, Sci-U: mid and upper (proximal) sciatic nerve. Med Ax, Rad Ax: axillary median and radial nerves.

162x121mm (300 x 300 DPI)

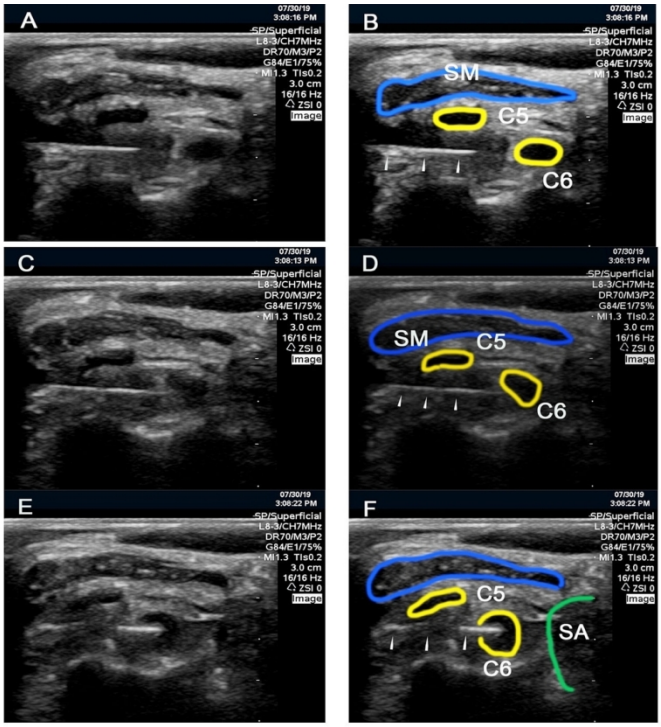


Fig 2. Representative ultrasound images of the right neck of cadaver 3 taken with a 3 to 8MHz transducer over the ventral C5 and C6 interscalene nerve roots. Images are cropped to a depth of 25mm. Image pairs are shown without (left) and with (right) annotation indicating needle position (3 arrowheads), C5 and C6 nerve roots outlined in yellow, scalenus medius (SM) outlined in blue, and scalenus anterior (SA) outlined in green. Images A and B show the needle tip approximately 3 mm from C6. Images C and D show the needle gently contacting epineurium. Images E and F show the needle tip within the subepineurium of C6 and the nerve has expanded secondary to subepineural injection.

159x119mm (300 x 300 DPI)

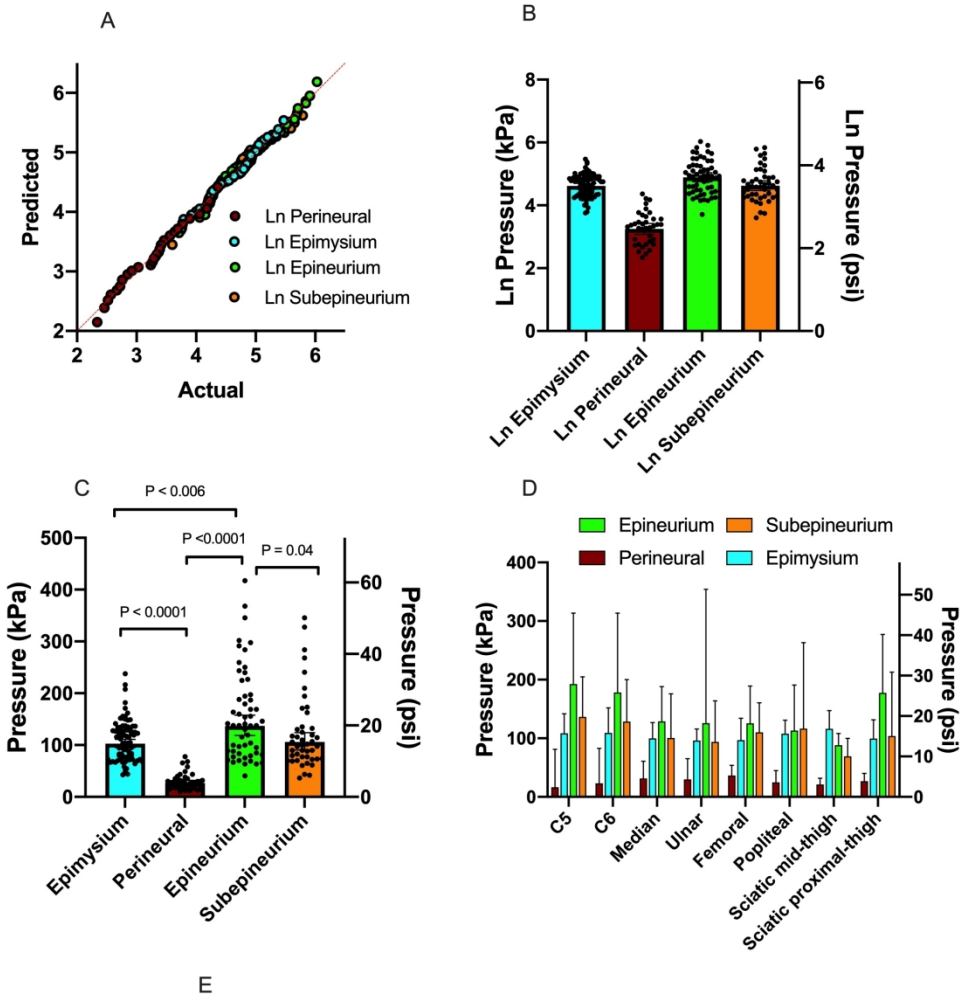


Fig 3. Injection peak pressure measurement from Study 1 with needle tip at fascia, at epineurium and in the subepineurium. Data was log-normal. Quantile-quantile (Q-Q) plot of logged pressure (A) follows a straight line and logged data show normal distribution (B). Pressure at epineurium greater than at other anatomical locations (C). Pressures follow rank order: epineurium > subepineurium = epimysium > perineural. Differences between pressures at anatomical interfaces over range of peripheral are consistent (D).

191x195mm (300 x 300 DPI)

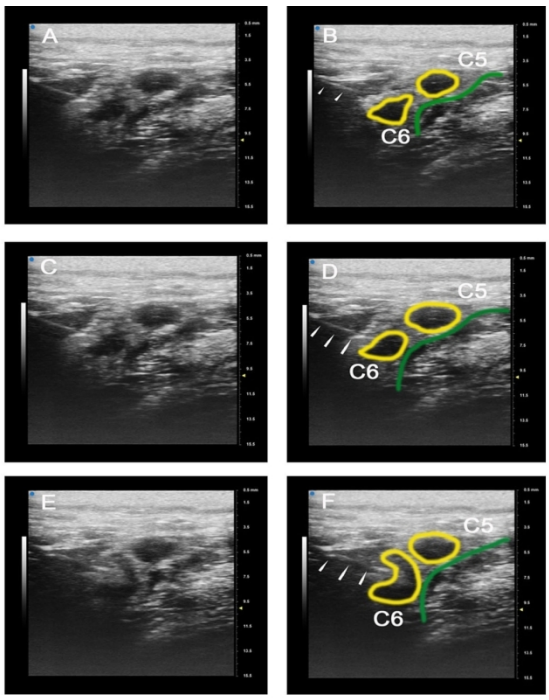


Fig 4. Ultrasound images of the right interscalene C6 ventral nerve root in soft embalmed Thiel cadaver no. 8, imaged using a 22- 45Mz microultrasound transducer. The scale on the right-hand side is from 0.5 to 15.5mm. Image pairs are show without (left) and with (right) annotation indicating needle position (arrowheads), C5 and C6 nerve roots outlined in yellow, and scalenus anterior muscle outlined in green. Images A and B shows the needle approaching the nerve. The tip is approximately 2 mm from the epineurium. Images C and D shows the needle touching the epineurium. Images E and F shows the needle tip indenting the epineurium.

159x119mm (300 x 300 DPI)

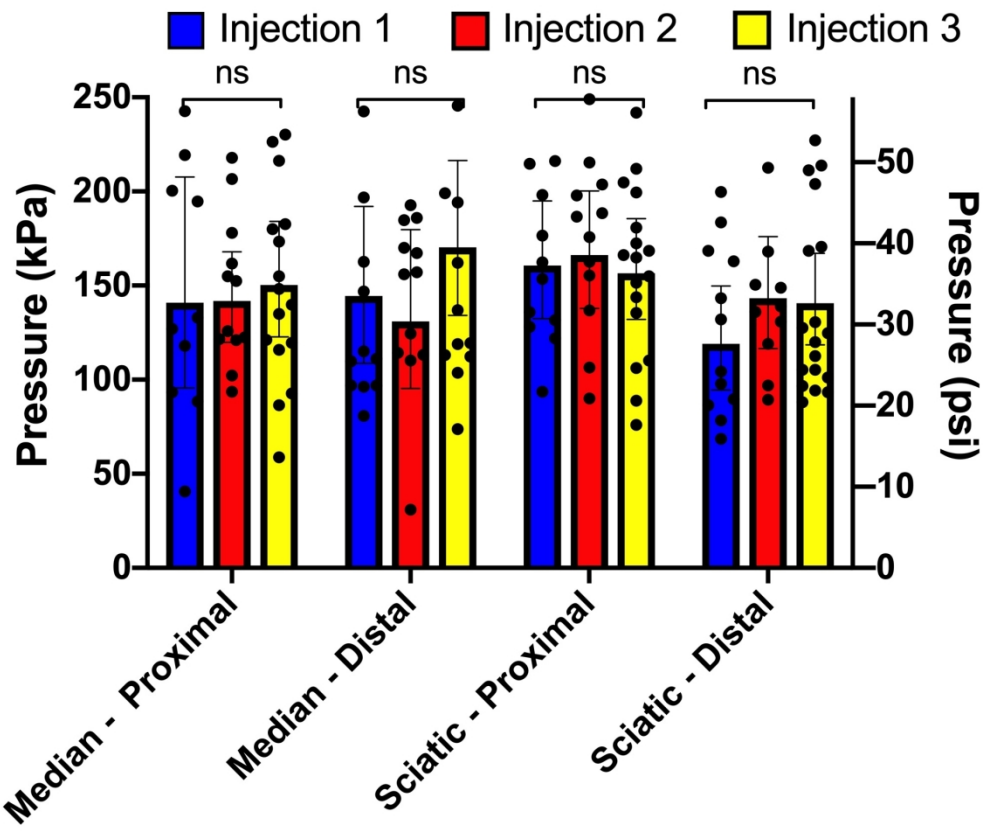
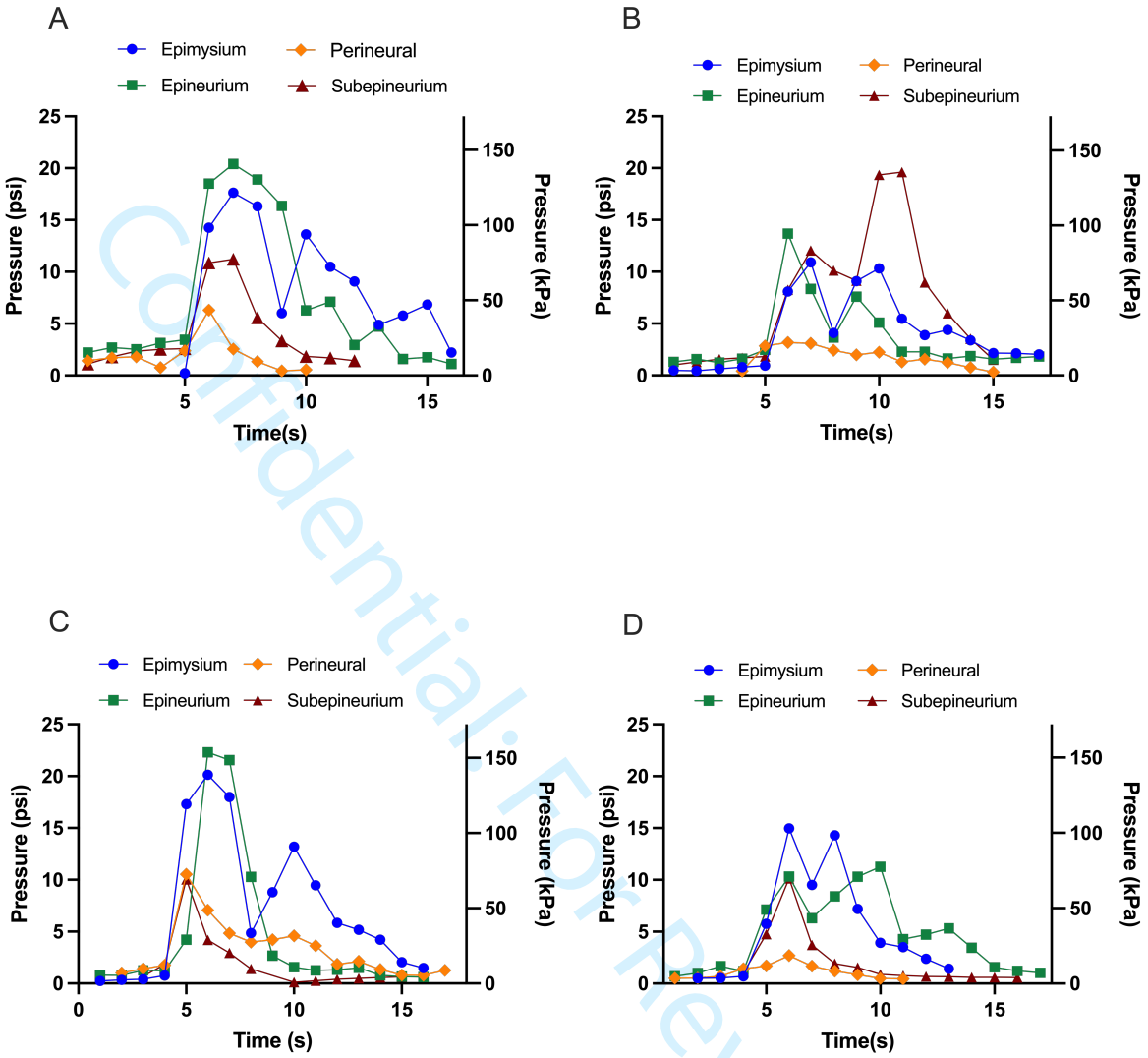


Fig 5. Injection peak pressures measurement epineurium. Study 2 pressure measurements acquired with flow rates of 1ml.min-1, 6ml.min-1 and 12ml.min-1 displayed with (A) linear scale and (B) following log conversion. Pressure measurements show a difference in pressure between a flow rate of 1ml.min-1 and 12ml.min-1, P = 0.005. Study 3 measurements (C) evaluate effect of repeated needle insertion using 12ml.min-1 flow rates. No difference in with repeated injection at each nerve or overall or injection site (all P-values > 0.05).

170x142mm (300 x 300 DPI)



Pressure/time plots at epimysium, in perineural tissue close to but not touching nerve, on epineurium and within subepineurium. Graphs are superimposed from beginning of injection. Sites of injection are: A, left mid-sciatic nerve; B, left femoral nerve; C, right ulnar nerve; and D, right popliteal nerve. Images A to D show typical variations in pressure recordings at tissue interfaces during needle advancement. Image A shows a rank order of pressure: epineurium > epimysium> subepineurium > perineural tissue. Image B shows higher pressures during subepineural injection. In image, C epineural and subepineural pressures are similar; and in Image D, pressures are highest in epimysium and similar at epineurium and in subepineurium.